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FINAL REPORT

BIOLOGICAL EFFECT OF ULTRAVIOLET RADIATION ON CATTLE: BOVINE OCULAR SQUAMOUS CELL CARCINOMA

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SUMMARY

This report summarizes the work done on the bovine ocular squamous cell carcinoma-ultraviolet radiation project from late February 1977 to December 31, 1977. It includes a description of the experimental set up and procedure used to irradiate cattle. Also included is a preliminary report of a UV-induced pre-neoplastic ocular growth.

A second part of this report summarizes an epidemiological study that shows that since 1950 the incidence of bovine ocular squamous cell carcinoma seen at slaughter has increased. This increase was real and not due to an increase in numbers of cattle.

The bovine ocular squamous cell carcinoma (cancer eye) study was begun in an effort to develop an animal model for the study of the biological effects of increased UV-B radiation that might follow modifications of the atmosphere.

The relationship between increased UV-B radiation and cancer eye has been assumed for many years. However, only in recent years has enough epizootiological data has been collected to give some credence to this hypothesis.[1] These data have been reviewed[1] (Table 1) and summarized.[2]

The geographic location of the high-incidence area of cancer eye involves two factors: time of exposure and intensity to solar radiation. At lower latitudes the period of exposure to sunlight is longer; the solar angle is greater, hence the solar radiation has less atmosphere to penetrate. At higher altitudes the decreased density of the atmosphere results in a greater intensity of solar radiation.

Lesions of cancer eye most frequently develop in unpigmented areas of the ocular region. Apparently pigment has a significant inhibitory effect against the development of lesion.

Cancer eye has been reported in almost every breed of cattle, but it is found much more frequently in the Hereford breed than in others.[3] Within the Hereford breed, increased susceptibility to cancer eye has been associated with certain strains of cattle which lack any pigment around the eye, the sclera and conjunctiva. Because the presence or absence of this pigment is a genetic trait in the Hereford breed, we can say that certain Herefords are hereditarily susceptible to cancer eye.

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The rate of occurrence of cancer eye in Herefords is based on actual observation and data collection, but the relationship of sunlight

or UV-B radiation to cancer eye is circumstantial. Therefore, it was decided to obtain more evidence as to the relationship between UV-B and cancer eye by actual irradiation studies in cattle.

In addition to the UV-B study, we studied existing data to determine the incidence of cancer eye in the U.S. cattle population.

Materials and Methods

Cattle--Six Hereford cattle that lacked pigment around the eyes were used. They were obtained from a purebred Hereford cattle herd[a] that had a history of cancer eye. The cattle were housed in 2 groups of 3 in an isolation barn that was air conditioned and temperature controlled. In each group of 3, two animals received UV-B radiation, and one animal served as the control. The controls were stanchioned in such a way that they received no UV radiation. The method of exposing the cattle to UV irradiation is shown in Photo. 1. One group of cattle was in a barn with 3 windows and a north exposure; the other group was housed similarly but had a south exposure. In addition to natural light, each barn was lighted by four 150-watt overhead bulbs. When being irradiated, the cattle faced away from the windows. The eyes of the cattle were observed daily.

Ultraviolet (UV-B) Irradiation and Source--The UV lights were Westinghouse FS-40 sunlamps mounted in a specular aluminum reflector fixture (Photo. 1). Each fixture was covered by a sheet of 5 mil cellulose acetate (CA)[b] which served to filter out UV of wavelengths below 280 nm. The CA sheets were pretreated for 6 hours with UV irradiation by FS-40 sunlamps, then used to cover the lamps which irradiated the cattle.

The CA sheets were replaced at intervals < 30 hours per sheet. The fixtures were hung about 102 cm from the plane of the cattle's eye (Photo. 1).

Initially a light fixture using 3 FS-40 bulbs was set up in a single fixture covered by CA. This was hung 61 cm from the eyes of the cattle. The change to the above described 4 lamp fixture was made early in the experiment to comply with the standardization that was desired in the Biological and Climatic Effect Research (BACER) program. At the time of the change, the time of exposure was increased 3x. This increased time of exposure was based on the relative dosage as determined by a IL 600 photometer.[c] The time change of irradiation was due to a change in distance and configuration of the lamp.

The cattle were exposed to the UV-B radiation daily (Table 3). The time varied from 7 minutes at the start of the experiment (February 28, 1977) to 150 minutes on December 31, 1977. The time of exposure was increased at irregular intervals but in such a way that the cornea was not directly injured by the UV-B radiation.

Measurement of Ultraviolet Radiation--The intensity of the UV radiation was measured in milliwatts/sq. meter/nanometer by a single-monochromator spectroradiometer[d] that was interfaced with a desk-top Hewlett-Packard 9815A programmable calculator. The calculator controls the operation of the spectroradiometer so that scanning is initiated on command and readings are recorded for each nanometer interval. The calculator prints out the wavelength and irradiance for each wavelength interval. The program was written by and is available from the Instrumentation Research Laboratory, USDA, Beltsville, MD.

Bacteriologic and Virologic Examination--The eyes were cultured periodically for Moraxella bovis and viruses; techniques previously described were used.[4] The main virus considered was cytopathic herpes virus because one group of investigators suggested that it might be involved in cancer eye of cattle.[e] Secretions from each eye of the 6 calves were collected with two sterilized cotton-tipped applicators 4 times before UV irradiation treatment began and once a month for 12 months. The nares were also cultured 4 times before irradiation began but were not cultured later. One applicator was used for the collection of eye secretions for bacteriologic examination and was placed in a sterilized dry screw-capped tube. The other applicator was used for virologic examination. Later, the secretions for bacteriologic examination were streaked on the surface of 5% blood agar plates and the plates were incubated at 37 C for 24 hours and at 25 C for 24 additional hours. Nasal secretions were handled in the same way as were the eye secretions. After incubation, the surface was observed for colonies of M. bovis.

The applicators with secretions for virologic examination were placed in Earle's balanced salt solution containing 0.25% lactalbumin hydrolysate, antibiotics (dihydrostreptomycin, 0.1 mg/ml; kanamycin, 0.1 mg/ml; and penicillin, 100 units/ml) and 10.0% (by volume) calf serum immediately after the sample was collected. Later, 0.1 ml of the suspension was used to inoculate each of 2 tubes of secondary embryonic bovine kidney (SEBK) cells. The cells were incubated at 37 C and observed each day for cytopathic effects (CPE) for 7 days.

Hematologic Examination--Venous blood was taken from the calves on their arrival at the National Animal Disease Center (NADC) and 9 times

during the study (Table 4). Blood was collected by venipuncture with evacuated glass tubes[f] containing EDTA for whole blood samples (used in determining total white blood cells (WBC) and differential WBC counts) and evacuated glass tubes free of any anticoagulant for serum collection. Total WBC counts were made with a Coulter counter.[g] The WBC differential counts were made after the cells were fixed and stained by a modification of the method[5] of May-Grundwald-Giemsa staining.

Incidence of Cancer Eye (Epithelioma) Determination--The data on cancer eye incidence and slaughter data was provided by Meat and Poultry Inspection (now Food Safety and Quality Service) of the USDA. These data were tabulated and then analyzed by 3RSSH method of Tukey.[6] For this report any squamous cell carcinoma involving the ocular region is known as an "epithelioma"; the two terms will be used interchangeably with "cancer eye."

Results

Bacteriologic Examination--*Moraxella bovis* was recovered from either the eyes or nares or both of 3 of 6 calves after the first examination. The infected calves were given oxytetracycline intravenously (I.V.) at a dosage of 7 mg/kg of body weight. When cultured 1 week later, one eye of each of 2 of the 3 previously infected calves was still infected; therefore, the calves were given oxytetracycline I.V. at a dosage of 11 mg/kg of body weight. *Moraxella bovis* was not cultured from the eyes or nares after the second treatment and the calves were presumed to be free of *M. bovis*.

Virologic Examination--Cytopathic changes were not observed in tubes of SEBK cells during the study and the eyes were presumed to be free of infection by cytopathic herpes virus.

Hematologic Data--Presented in Table 4.

Gross Anatomical Changes--All mucosa surfaces of the eye were highly inflamed. The sclera and conjunctiva of the cattle developed various degrees of plaque formation and papilloma formation that appeared after 16 weeks of radiation. The type lesions observed were considered typical of early cancer eye (Photo. 2).

Histopathological Changes--A biopsy taken from one animal on October 31, 1977 (after 36 weeks of radiation) revealed metaplastic changes consistent with preneoplasia including diffuse epithelial hyperplasia with acanthosis and presence of foci of clusters of typical squamous cells in adjacent dermis.

The dermis was markedly edematous, and its vasculature and collagen fibers were distorted and irregular. Many aggregates of small lymphocytes were distributed throughout the dermis, and small foci of neutrophils were present in many areas. Accumulations of eosinophilic proteinaceous material, representing intraepithelial edema (blister formation), were present between the stratum germinativum and stratum spinosum in two areas.

Squamous cells in dermal clusters and its overlying epithelium appeared atypical and moderately anaplastic, e.g., they were large, and pleomorphic and had large, irregular nuclei with large nucleoli. The dermis surrounding these squamous cell clusters (or epithelial pearls) was edematous and contained fibrin and neutrophils.[h] (Photo. 3 and 4.)

Incidence of Cancer Eye (Epitheliomas)--The preliminary results of the survey are shown (Table 5). There has been a 5x increase in total epitheliomas recorded at the slaughter plants. When the total epitheliomas were adjusted to consider the increase in number of animals slaughtered, the incidence of epitheliomas increased about 2x (Table 5).

The incidences of epitheliomas were compared for 3 years with 3 other causes for condemnation (Table 6). When only animals that were condemned on the "kill floor" at the abattoir were considered, condemnation due to ephtheliomas increased greater than those due to other causes. After the number condemned was adjusted for the increase in total slaughter from 1955 to 1975, only condemnations due to epitheliomas increased; condemnations due to other causes decreased.

Discussion

The experimental results in the present study represent the first successful attempt at producing neoplastic changes in a short period by UV-B irradiation and normal animals exclusively. Previous work[7] with UV-B irradiation involved the use of either genetic variants or various carcinogenic chemicals or both. Various forms of skin cancer were produced in these animals, but it was difficult to know whether the UV-B or the chemical produced the lesion.

Most work in which UV-B irradiation was used involved the use of mercury lamps that have a very strong emission at 254 nm and a damaging effect on DNA. In this present study this strong wavelength emission was eliminated by the use of 5 mil of CA which adsorbed wavelengths below 280 nm. This situation is thought to be much closer to the natural

UV-B which penetrates the atmosphere and reaches the surface of the earth. This filter also eliminates the strong UV band at 254 nm which in itself is very damaging to cells because of effect on DNA. Further evidence that the UV-B was the sole inducer of the lesions in the present study is the fact that cytopathic herpes viral agents and _M. bovis_ were not isolated.

Although epithelioma at the abattoir seemed to increase, more information is needed because this apparent increase might be due to an increased awareness by the meat inspectors, resulting in a spurious increase in cancer eye incidence. It might be due to a combination of both an actual increase and increased awareness by the inspectors. We favor the hypothesis that there is a real increase because our investigation indicated that epitheliomas were the only cause for condemnation that actually increased out of the several diseases studied. We think that the increased awareness was responsible for the large increase in total epitheliomas detected at slaughter but that the increase in the incidence of condemned animals was due to a real increase in severe cases of epithelioma. Further evidence supporting this hypothesis is the fact that when we were looking for a cause of increased condemnations, we related the incidence of epitheliomas with solar activity.[8] We[1] plotted with a 5 year latent period (from time of first exposure to time of clinical signs) epitheliomas against solar activity; they matched quite well (Fig. 2). Because cancer eye and solar radiation appeared to be interrelated, this finding should not be so surprising. A delay in appearance would correspond to the time from first exposure to development of clinical signs.

This study to date has shown that UV-B irradiation is a probable cause of cancer eye and that increased UV-B radiation could be expected to increase both the total cases of cancer eye in susceptible animals as well as an increase in the severity of this disease.

Footnotes

[a]Meat Animal Research Center, USDA, Clay Center, Nebraska.

[b]Celanese Plastics, 26 Main Str., Chatham, New Jersey.

[c]International Light, Inc., New Buryport, Massachusetts.

[d]Model 1741, Optronic Lab., Inc., 7676 Fenton Street, Silver Spring, Maryland.

[e]Anson M: Bovine ocular squamous cell carcinoma: In vitro investigation of a viral etiology. Abstracts No. 83, page 15, 57th Ann. Mtg. of Research Workers in Animal Diseases. Chicago, Illinois, Nov. 29-30, 1976.

[f]Vacutainer, Becton-Dickinson, Div. of Bectan, Dickinson and Company, Rutherford, New Jersey.

[g]Coulter Counter, Model ZB_1, Coulter Electronic, Inc., Hialiah, Florida.

[h]Description and interpretation by N. F. Cheville, Pathological Laboratory, NADC, Ames, Iowa.

[i]Statistical operations performed by G. D. Booth, NADC, Ames, Iowa.

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8. Special Committee for Solar Terrestrial Physics, National Academy of Science: Solar-Terrestrial Physics and Meteorology: A working document. Washington, DC, 1975.

TABLE 1—Epidemiological Evidence for Relationship of Cancer Eye and Sunlight (Anderson, 1970)

Criterion	Level of sunlight	No. of animals	Age adjusted frequency of cancer eye (%)
Latitude	Low	3445	3.6
	Med	361	7.8
	High	1154	9.2
Altitude	Low	670	4.8
	Med	3909	5.1
	High	381	9.7
Hours of sunlight	Low	3445	3.6
	Med	823	5.6
	High	692	11.7

TABLE 2—Irradiance from Four FS-40 Lamps at 100 cm With and Without Cellulose Acetate Filters per Nanometer

Filter	Condition of filter	Irradiance 280-320 milliwatts/sq. meter
None	-	11.3346
5 mil CA	New	6.5262
5 mil CA	Used 5 hr	5.4734
5 mil CA	Used 25 hr	4.3178

TABLE 3--Exposure Time to UV-B Irradiation

Length of exposure	Number of days	Total time (minutes)
7	2	14
10	5	50
A* 15	2	30
20	26	520
25	18	450
30	28	840
		1940
90	56	5040
105	42	4410
B** 120	42	5040
135	70	9450
150	15	2250

*A= 3 FS-40 bulbs in single fixture, 60 cm from plane of eyes; Irradiance was not measured by spectroradiometer but was approximately 3x greater than B when measured by IR-600.

**B= 2 FS-40 bulbs, 2 fixtures, 60 cm apart, 100 cm from plane of eyes; Irradiance = 4.8956 milliwatts/sq. meter/nm.

TABLE 4—Total White Blood Cell (WBC) Counts and Selected (Eosinophils, Neutrophils, Lymphocytes, and Monocytes) Differential WBC Counts of UV-B Irradiated and Nonirradiated Calves During Nine 12 Months Study*

Calf No.	Date	Eosin	Neut	Lymph	Mono	Total WBC	Calf No.	Date	Eosin	Neut	Lymph	Mono	Total WBC
**2042	2-28	1	13	85	1	8542	**2698	2-28	4	21	68	3	8754
	3-23	5	11	81	2	10400		3-23	2	11	84	3	10600
	5-5	1	13	84	2	10300		5-5	4	14	79	3	12400
	6-15	2	15	81	2	11600		6-15	3	12	83	2	11000
	7-13	4	18	72	7	11100		7-13	4	22	68	6	11200
	8-18	3	9	84	4	10600		8-18	5	22	70	3	12700
	9-28	3	12	83	2	11000		9-28	6	17	75	2	13200
	11-16	2	7	87	4	10300		11-16	5	21	70	4	10800
	12-21	3	4	91	2	6909		12-21	2	15	80	3	9614
2369	2-8	2	34	66	5	5999	2709	2-8	4	27	66	4	6234
	3-23	2	22	73	4	6968		3-23	3	16	82	2	6789
	5-5	1	13	84	2	6497		5-5	7	28	60	5	9467
	6-15	4	10	85	1	6742		6-15	4	22	70	3	7939
	7-13	4	9	85	4	6629		7-13	5	12	75	6	8435
	8-18	4	20	71	5	7980		8-18	5	12	79	5	8607
	9-28	9	14	70	7	8038		9-28	6	18	73	3	9422
	11-16	4	13	79	4	8297		11-16	3	16	76	5	6683
	12-21	1	13	80	6	6053		12-21	6	13	79	2	7818
2623	2-8	3	26	64	6	8303	2713	2-8	9	17	71	3	12200
	3-23	5	16	77	2	9991		3-23	5	17	75	4	7499
	5-5	4	20	74	2	10800		5-5	8	18	71	4	9491
	6-15	4	28	67	1	11700		6-15	8	16	74	2	7663
	7-13	7	16	71	6	11300		7-13	6	29	59	6	9184
	8-18	3	17	77	3	13800		8-18	8	13	73	6	8965
	9-28	4	13	78	5	12400		9-28	4	12	79	5	9537
	11-16	5	17	76	2	11000		11-16	5	22	67	6	9795
	12-21	3	13	82	2	9490		12-21	12	9	77	2	11100

*Basophils and myelocytes counts were determined to be less than 1% and therefore are not recorded.

**Nonirradiated control.

TABLE 5--Cattle Slaughtered with Epitheliomas

Year	Total slaughtered $\times 10^6$	Total epithelioma $\times 10^3$	Epithelioma as 1/100 of percentage total slaughtered
1950	13.1	24.5	19
1951	12.6	24.7	20
1952	12.1	28.0	23
1953	15.2	30.0	20
1954	18.5	36.0	20
1955	18.7	35.4	19
1956	19.7	38.1	19
1957	20.1	46.9	23
1958	18.6	47.4	26
1959	17.3	39.7	23
1960	18.5	59.1	32
1961	19.9	69.6	35
1962	20.2	65.1	32
1963	20.9	75.0	36
1964	23.2	79.8	34
1965	25.8	85.8	33
1966	27.4	85.2	31
1967	27.9	77.3	28
1968	28.1	87.7	31
1969	30.2	109.9	36
1970	30.9	136.0	44
1971	31.0	102.7	33
1972	31.7	109.0	34
1973	31.6	123.8	39
1974	30.9	147.3	48
1975	34.8	141.7	41
1976	36.8	131.1	36

TABLE 6--Comparison of 3 Condemnation Causes and Epithelioma for 3 Different Years

Causes of Condemnation		1955	1965	1975
Pneumonia	T	12,407(100*)	61,692(497)	152,063(1226)
	Adj.**		45,031(362)	81,754(659)
	C	12,407(100)	9,654(78)	12,134(98)
	Adj.		7,047(57)	6,524(53)
Abscesses	T	29,158(100)	415,709(1425)	508,475(1744)
	Adj.		303,437(1040)	273,373(934)
	C	9,675(100)	9,154(95)	11,445(118)
	Adj.		6,682(69)	6,153(63)
Lymphona	T & C	2,680(100)	4,616(172)	4,160(158)
	Adj.		3,369(126)	2,237(83)
Epithelioma	T	34,394(100)	85,841(243)	141,727(400)
	Adj.		62,658(177)	76,197(215)
	C	4,077(100)	7,139(175)	21,491(527)
	Adj.		5,211(128)	11,554(283)
Total Slaughter		18,728,579 (100)	25,803,948 (137)	34,825,463 (186)

T = Total cases condemned + retained.

C = Total condemned.

*Percent (%) of 1955 value.

**Adjusted to rate of increase from 1955 to 1975 for total slaughter.

Photographs

Photo. 1--Arrangement of cattle and UV lamps.

Photo. 2--Early gross changes on 3rd eyelid and corneoscleral junction after UV-B irradiation.

Photo. 3--Tissue section through 3rd eyelid. 25 X.

Photo. 4--Same as Photo. 3, but 100 X.

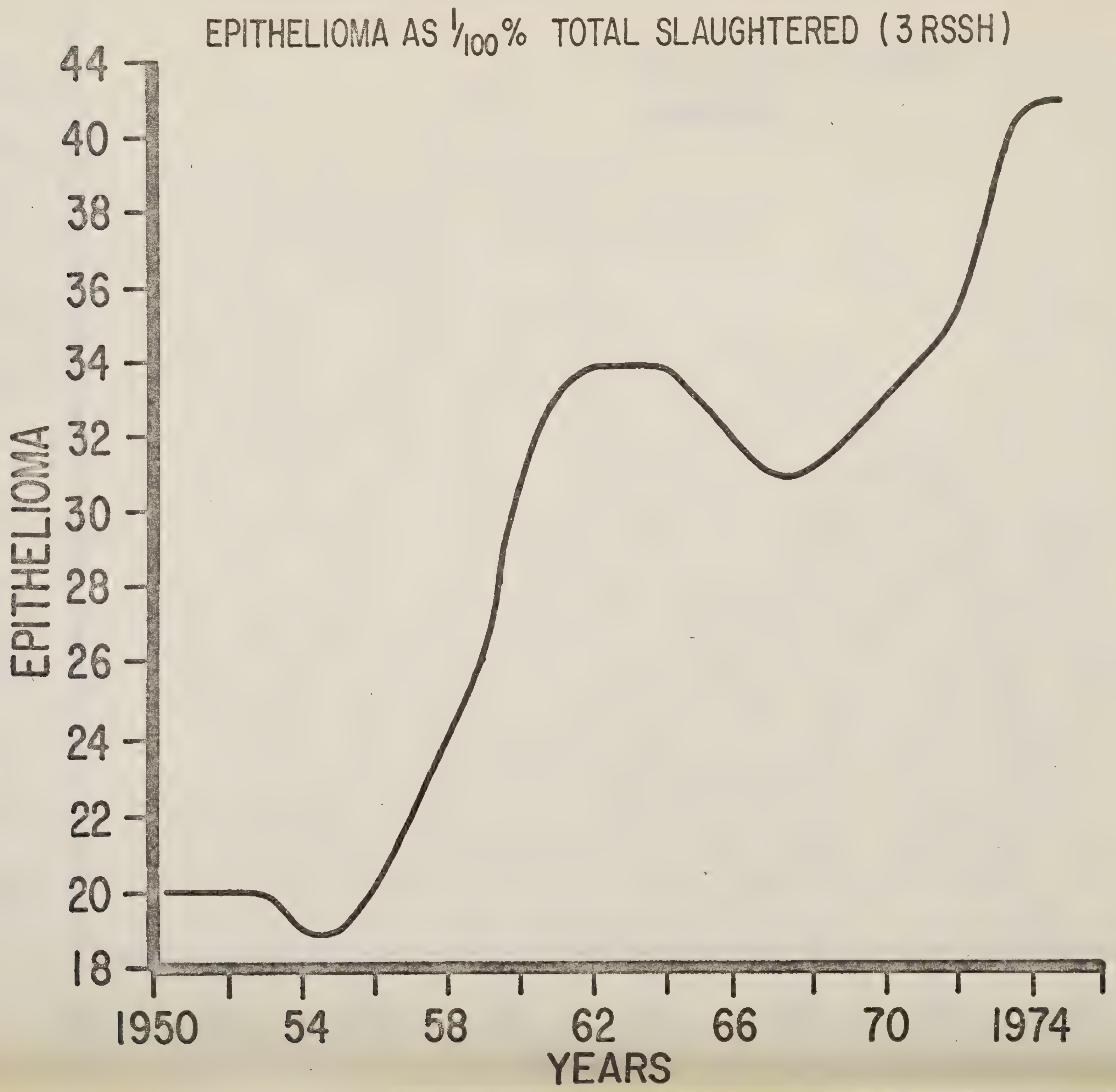
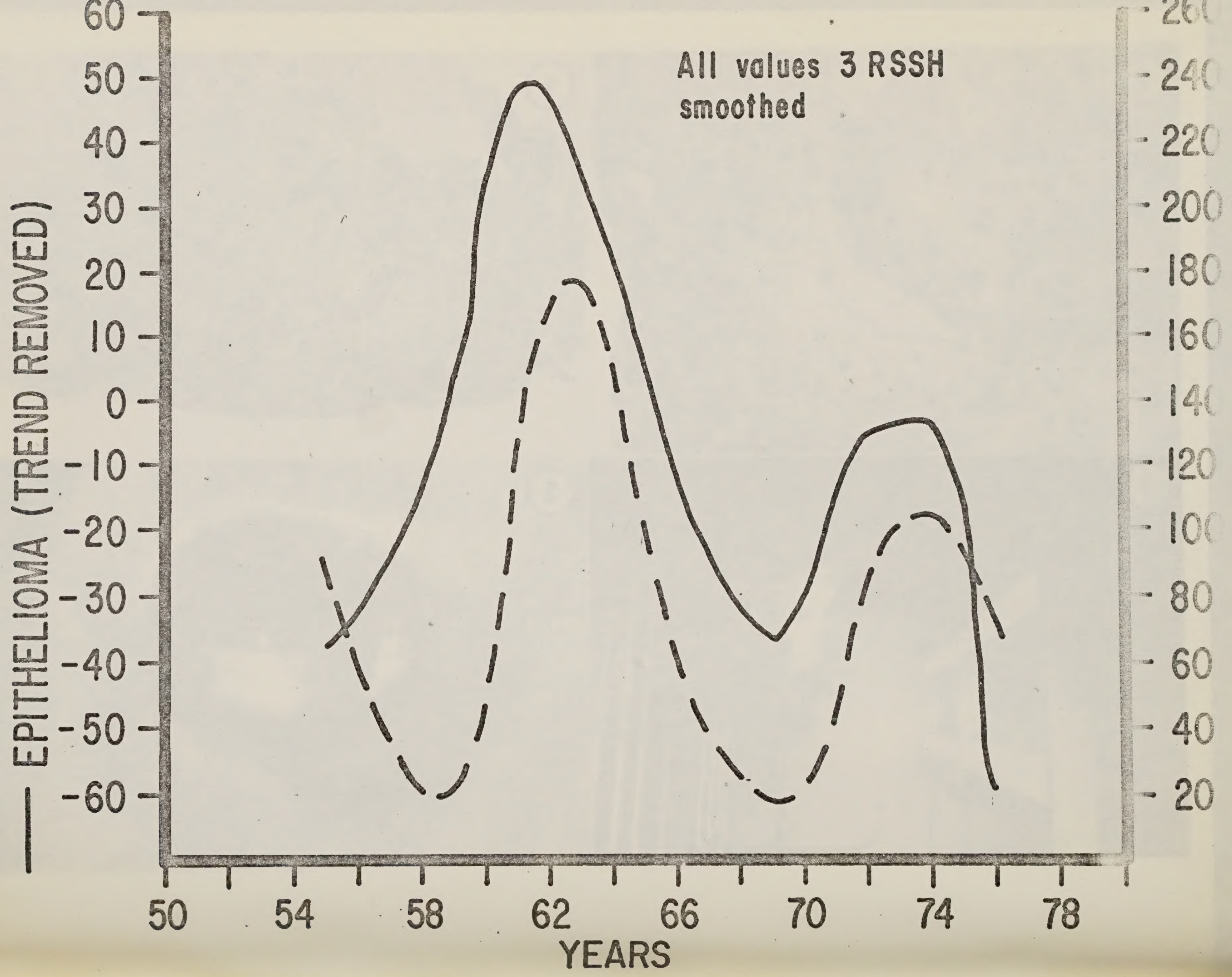
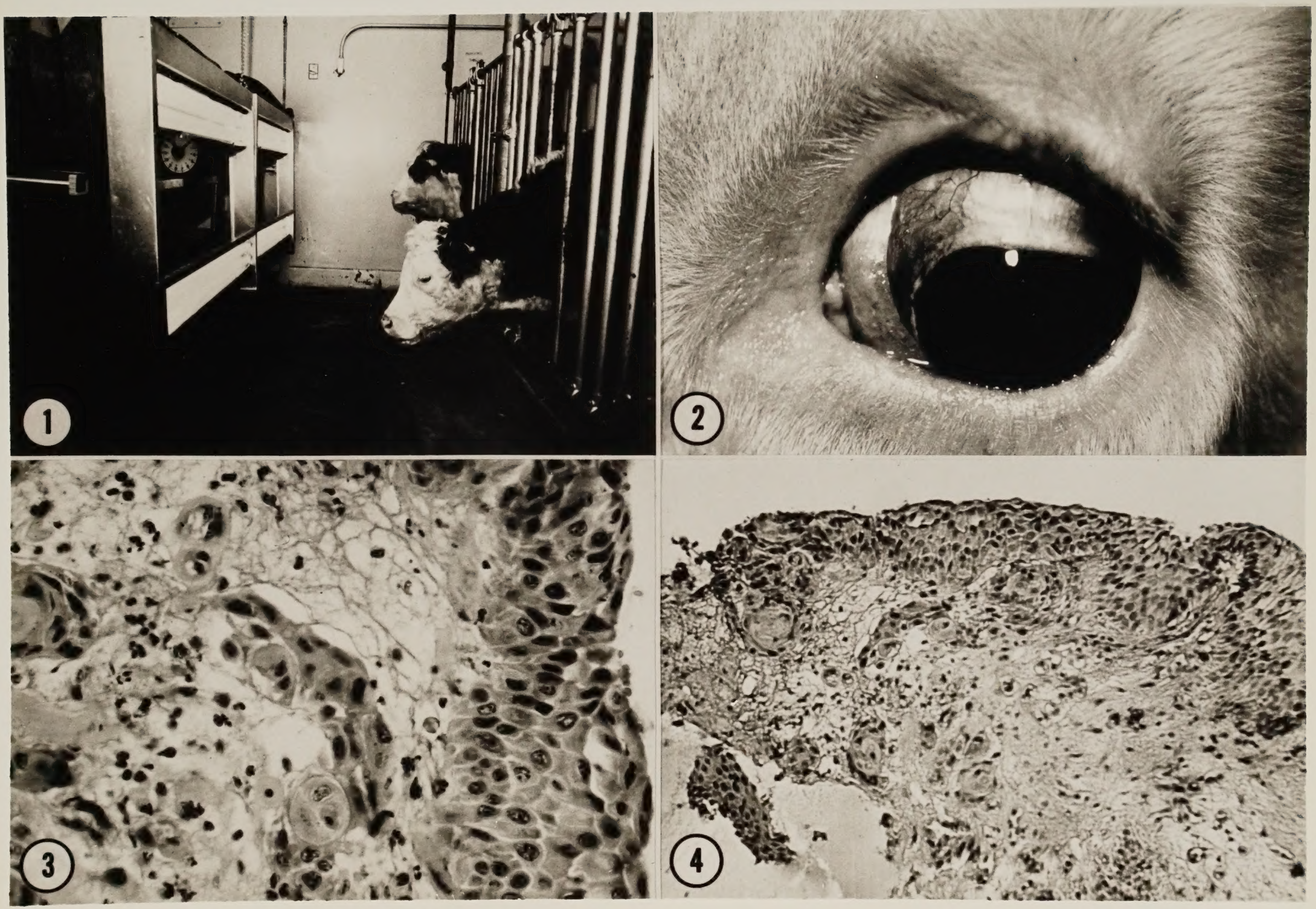

EPITHELIOMA AS 1/100% TOTAL SLAUGHTERED (3 RSSH)
EPITHELIOMA
44
40
38
36
34
32
30
28
26
24
22
20
18
1950
54
58
62
66
70
1974
YEARS

FIG. 1



EPITHELIOMA (TREND REMOVED)
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
All values 3 RSSH smoothed
50
54
58
62
66
70
74
78
YEARS
20
40
60
80
100
120
140
160
180
200
220
240
260
SOLAR ACTIVITY

FIG. 2



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